

RESEARCH ARTICLE

Association Between Three eNOS Polymorphisms and Cancer Risk: a Meta-analysis

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Abstract

Polymorphisms in the endothelial nitric oxide synthase (eNOS) gene may influence the risk of cancer, but the results are still debatable. Therefore, we performed a systematic review to provide a more complete picture and conducted a meta-analysis to derive a precise estimation. We searched PubMed, EMBASE, EBSCO, Google Scholar and China National Knowledge Infrastructure (CNKI) databases until April 2014 to identify eligible studies. Thirty-one studies with cancer patients and controls were included in the meta-analysis. Overall, the pooled analysis revealed that the T-786C polymorphism was significantly associated with increased cancer risk under multiple genetic models (C vs T: OR=1.135, 95% CI=1.048-1.228; CC vs TT: OR=1.278, 95% CI=1.045-1.562; TC vs TT: OR=1.136, 95% CI=1.023-1.261; CC+TC vs TT: OR=1.159, 95% CI=1.047-1.281; CC vs TC+TT: OR=1.204, 95% CI=1.003-1.447). G894T was associated with significant risk for females (TT vs GG: OR=1.414, 95% CI=1.056-1.892; TT vs GT+GG: OR=1.356, 95% CI=1.108-1.661) and for breast cancer (T vs G: OR=1.097, 95% CI=1.001-1.203; TT vs GG: OR=1.346, 95% CI=1.012-1.789; TT vs GT+GG: OR=1.269, 95% CI=1.028-1.566). Increased susceptibility was revealed for prostate cancer with 4a/b (ba vs bb: OR=1.338, 95% CI=1.013-1.768; aa+ba vs bb: OR=1.474, 95% CI=1.002-2.170). This meta-analysis indicated that the eNOS T-786C polymorphism is associated with elevated cancer risk; the G894T polymorphism contributes to susceptibility to breast cancer and cancer generally in females; and the 4a/b polymorphism may be associated with prostate cancer risk.

Keywords: Endothelial nitric oxide synthase - polymorphism - cancer - systematic review - meta-analysis

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Introduction

Cancer is a major public health problem and it is one of the leading causes of death worldwide (Jemal et al., 2011). The global burden of cancer is ever-increasing in both economically developed countries and developing countries (Are et al., 2013). Tremendous efforts have been made to unravel the underlying mechanism of cancer, with the aim to develop optimal prophylactic and therapeutic strategies. The mechanism of developing cancer is still unclear. People generally agree that complex environmental factors and interindividual genetic susceptibility may contribute to cancer development (Perera, 1997). Substantial evidence shows that genetic susceptibility has a significant role in an individual's risk of developing cancer (Dong et al., 2008).

Nitric oxide (NO) is a short-lived, pleiotropic molecule that play complicated roles in tumor biology. NO is generated by three isoforms of NO synthase (NOS): neuronal (nNOS/NOS1), inducible (iNOS/NOS2) and endothelial (eNOS/NOS3) (Burke et al., 2013). Endothelial nitric oxide synthase (eNOS) is constitutively expressed

in endothelial or epithelial cells, including a variety of tumours (Ying et al., 2007). The gene encoding for eNOS is located on chromosome 7q36 and contains 26 exons in humans (Marsden et al., 1993), and polymorphisms in the eNOS gene have been widely studied. Till now, a number of polymorphisms and mutations within the eNOS gene have been identified, with the most studied being G894T, T-786C and 4a/b polymorphisms. The G894T (Glu298Asp, rs1799983) polymorphism corresponds to a Glu-Asp change at codon 298 in exon 7. T-786C (rs2070744) polymorphism is a point mutation of thymine to cytosine at nucleotide -786 in the 5'-flanking region of the eNOS gene which could result in a significant reduction in eNOS gene promoter activity and reduce serum NO level significantly (Nakayama et al., 1999). 4a/b polymorphism is a variable number of tandem repeats (VNTR, 27nt) in intron 4 accounts influencing basal plasma NO generation (Wang et al., 1997). Many studies have investigated the influence of eNOS polymorphisms on cancer risk, whereas the results remained conflicting.

Therefore, we conducted comprehensive literature search with the aim to provide an overview of studies

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focusing on the relationship between eNOS polymorphisms and cancer risk. Meanwhile, we performed a meta-analysis combining all available data to estimate the potential associations of eNOS polymorphisms with cancer risk.

Materials and Methods

Literature search

We searched the articles using the search terms “NOS,” “eNOS,” “NOS3,” “polymorphism (s),” “genotype,” “variant,” “carcinoma,” “cancer,” “tumor,” and “malignancy” in PubMed, EMBASE, EBSCO, Google Scholar and China National Knowledge Infrastructure (CNKI) databases without a language limitation, and the last search updated on 5 April 2014. We evaluated all associated publications to retrieve the most eligible literatures. Their reference lists were hand-searched to find for other relevant publications. Only published studies with the full text articles were included. If the same patient population was included in several publications, the most recent or complete study was used in this meta-analysis.

Inclusion criteria

Studies were included in the meta-analysis if (1) it investigated the association between eNOS polymorphism and cancer risk; (2) the design was case-control study or nested case-control study; (3) data regarding genotype distributions were sufficient to calculate the odds ratio (OR) and its corresponding 95% confidence interval (CI). When studies with overlapping subjects were considered eligible, only the one with a larger number of patients was included. If the data regarding genotype distribution was insufficient, the effort was made to contact its corresponding author.

Data extraction

Two reviewers (Xun Wu and Zifeng Wang) extracted the following information from each included study independently and in duplicate: first author's name, publication year, country, source of the study population, cancer type, polymorphisms studied, source of control, number of cases and controls, match criteria, genotype distribution in cases and controls, and whether or not the genotype distributions among controls were in accordance with Hardy-Weinberg equilibrium (HWE). A third reviewer (Yin Xu) was consulted to reach a consensus if any discrepancy occurred.

Statistical analysis

The summary ORs and their corresponding 95 % CI were calculated to assess the strength of the association between eNOS polymorphism and cancer risk. Z-test was performed to determine the statistical significance of pooled ORs, and $p < 0.05$ was considered significant. Cochran's Q test and the I² statistic were used to measure heterogeneity across the included studies. A P value of more than 0.05 for the Q test indicated a lack of heterogeneity, and the fixed-effects model was subsequently used to calculate the summary ORs. Otherwise, the random-effects model was applied. Publication bias was estimated by visually assessing the asymmetry of Begg's funnel

plot. Furthermore, Egger's test was performed to provide quantitative evidence for the checking of publication bias. Sensitivity analysis was also performed by sequentially omitting individual study to check the stability of the result. $P < 0.05$ was considered statistically significant. All the statistical analysis was performed using STATA12.0 (STATA Corporation, College Station, TX, USA).

Results

Identification and characteristics of included studies

The process of study selection was summarized in the flow diagram (Figure 1). Finally, 31 studies (29 in English and 2 in Chinese) with a total of 9310 cases and 9786 controls were included in this study. It should be noted that Lee et al. studied polymorphisms in Caucasians and African-Americans respectively (Lee et al., 2009). Therefore, we treated them as separate data sets during our analysis. The characteristics of these eligible studies were summarized in Table 1. The most commonly investigated polymorphism was G894T, followed by 4a/b and T-786C, which were reported in 27 (Hefler et al., 2002; Medeiros et al., 2002; Ghilardi et al., 2003; Riener et al., 2004; Conde et al., 2006; Hefler et al., 2006; Lu et al., 2006; Marangoni et al., 2006; Royo et al., 2006; Lee et al., 2007; Chen et al., 2009; Funke et al., 2009; Harman et al., 2009; Lee et al., 2009; Li et al., 2009; Yeh et al., 2009; Chen et al., 2010; Unal et al., 2010; Zintzaras et al., 2010; Ozturk et al., 2011; Ryk et al., 2011; Arikan et al., 2012; Brankovic et al., 2013; Jang et al., 2013; Safarinejad et al., 2013; Verim et al., 2013; Ziaei et al., 2013), 14 (Hefler et al., 2002; Medeiros et al., 2002; Riener et al., 2004; Hefler et al., 2006; Lu et al., 2006; Yeh et al., 2009; Unal et al., 2010; Zintzaras et al., 2010; Ozturk et al., 2011; Sanli et al., 2011; Amasyali et al., 2012; Jang et al., 2013; Safarinejad et al., 2013; Yuan et al., 2013) and 11 (Ghilardi et al., 2003; Conde et al., 2006; Lu et al., 2006; Lee et al., 2007; Marangoni et al., 2008; Yeh et al., 2009; Unal et al., 2010; Ryk et al., 2011; Brankovic et al., 2013; Jang et al., 2013; Safarinejad et al., 2013) studies, respectively. There were 9 studies for prostate cancer, 7 studies for breast cancer, 5 studies for colorectal cancer, 3 studies for bladder cancer, 7 studies for other 5 different cancers and adrenal incidentaloma. Among those 31 studies, there were 22 Caucasian, 6 Asian, 3 fixed and 1 African American studies, respectively. A summary of the meta-analysis findings of the association

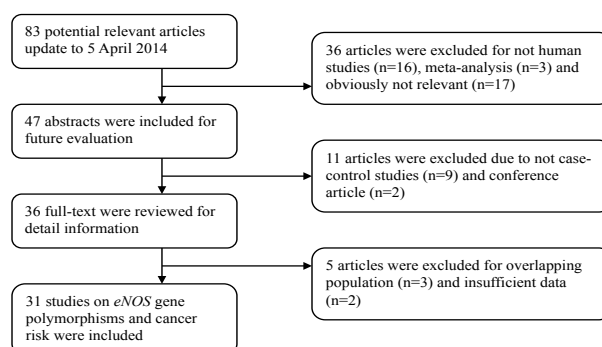


Figure 1. Flow Chart of Literature Search and Selection

between eNOS gene polymorphisms and cancer risks is provided in Table 2.

The association between G894T polymorphism and cancer risk

Data from 25 case-control studies and 2 nested case-control studies comprising 7775 cases and 7817 controls were pooled together for analysis of the G894T polymorphism. Significantly increased cancer risks were found for TT vs GG in studies with matched controls enrolled (OR=1.219, 95%CI=1.019-1.457) and females (OR=1.414, 95%CI=1.056-1.892). Similar situations were found for TT vs GT+GG in studies with females (OR=1.356, 95%CI=1.108-1.661), Asians (OR=2.103, 95%CI=1.133-3.903) and mixed population (OR=1.648, 95%CI=1.056-2.571). In subgroup analysis by cancer type, we found

significantly increased breast cancer susceptibility in three models (T vs G: OR=1.097, 95%CI=1.001-1.203; TT vs GG: OR=1.346, 95%CI=1.012-1.789; TT vs GT+GG: OR=1.269, 95%CI=1.028-1.566) (Figure 2).

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by sequential omission of each eligible study. The analysis results showed that the pooled ORs were not significantly affected by any individual study (Figure 3a), thus indicating a robust result of the analysis.

Begg's funnel plot was constructed to evaluate the publication bias of literatures on cancer. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Figure 4a). Furthermore, Egger's test provided statistical evidence for the lack of publication bias ($t=0.61, p=0.548$).

Table 1. Characteristics of Studies Included in this Meta-Analysis

| Author | Country | Ethnicity Gender | Polymorphisms investigated | Cancer type | Source of control | Genotyping method | Case/control | Matching criteria |
|-------------------------|----------|------------------|----------------------------|--------------------------|-----------------------|------------------------|--------------|--------------------------------|
| Harman et al. 2009 | Turkey | Caucasian | G894T | Adrenal incidentaloma | Hospital-based | TaqMan | 50/30 | NA |
| Ryk et al. 2011 | Sweden | Caucasian | G894T,T-786C | Bladder cancer | Population-based | TaqMan, sequencing | 262/150 | Geographical area, age |
| Amasyali et al. 2012 | Turkey | Caucasian | 4a/b | Bladder cancer | Hospital-based | PCR | 123/201 | Age, gender |
| Verim et al. 2013 | Turkey | Caucasian | G894T | Bladder cancer | Population-based | PCR-RFLP | 66/88 | Age, gender |
| Ghilardi et al. 2003 | Italy | Caucasian | G894T,T-786C | Breast cancer | Population-based | PCR | 71/91 | Age |
| Hefler et al. 2006 | Germany | Caucasian | G894T,4a/b | Breast cancer | Hospital-based | PCR | 269/244 | Geographical area, age |
| Lu et al., 2006 | America | Caucasian | G894T,T-786C, 4a/b | Breast cancer | Hospital-based | PCR-RFLP, sequencing | 421/423 | Age |
| Royo et al. 2006 | Spain | Caucasian | G894T | Breast cancer | Population-based | Sequencing | 440/321 | Age |
| Lee et al. 2007 | Korea | Asian | G894T,T-786C | Breast cancer | Mixed | SNP-JTMM | 1348/944 | Age, education |
| Li et al. 2009 | America | Mixed | G894T | Breast cancer | Population-based | TaqMan | 428/422 | Age, ethnicity |
| Zintzaras et al. 2010 | Greece | Caucasian | G894T,4a/b | Breast cancer | Hospital-based | Sequencing | 306/131 | NA |
| Conde et al. 2006 | Spain | Caucasian | G894T,T-786C | Colorectal cancer | Population-based | FRET, pyrosequencing | 360/550 | NA |
| Funke et al. 2009 | Germany | Caucasian | G894T | Colorectal cancer | Population-based | Pyrosequencing | 632/604 | Age, gender, geographical area |
| Yeh et al. 2009 | Taiwan | Asian | G894T,T-786C, 4a/b | Colorectal cancer | Population-based | PCR | 726/736 | Age, gender |
| Arikan et al. 2012 | Turkey | Caucasian | G893T | Colorectal cancer | Hospital-based | PCR-RFLP | 84/99 | NA |
| Jang et al. 2013 | Korea | Asian | G894T,T-786C, 4a/b | Colorectal cancer | Population-based | PCR | 528/509 | Age |
| Ozturk et al. 2011 | Turkey | Caucasian | G894T,4a/b | Endometrial cancer | Hospital-based | PCR | 89/60 | NA |
| Chen et al. 2010 | China | Asian | G894T | Gastric cancer | Population-based | PCR-RFLP | 324/200 | Age, gender |
| Unal et al. 2010 | Turkey | Caucasian | G894T,T-786C, 4a/b | Gastric cancer | Population-based | PCR | 50/98 | NA |
| Yuan et al. 2013 | China | Asian | 4a/b | Hepatocellular carcinoma | Hospital-based | PCR, sequencing | 293/384 | Age, gender |
| Hefler et al. 2002 | Austria | Caucasian | G894T,4a/b | Ovarian cancer | Population-based | Pyrosequencing, PCR | 130/133 | Age |
| Medeiros et al. 2002 | Portugal | Caucasian | G894T,4a/b | Prostate cancer | Population-based | PCR | 125/153 | Age |
| Marangoni et al. 2006 | Brazil | Mixed | G894T | Prostate cancer | BPH | PCR-RFLP | 89/60 | NA |
| Marangoni et al. 2008 | Brazil | Mixed | T-786C | Prostate cancer | BPH | PCR-SSCP, sequencing | 83/94 | NA |
| Chen et al. 2009 | China | Asian | G894T | Prostate cancer | BPH | PCR-RFLP | 78/88 | Age |
| Lee et al. 2009 | America | Caucasian | G894T | Prostate cancer | Population-based | TaqMan | 1213/1433 | Age, ethnicity |
| Sanli et al. 2011 | Turkey | African | G894T | Prostate cancer | Population-based | TaqMan | 107/409 | Age, ethnicity |
| Sanli et al. 2011 | Turkey | American | 4a/b | Prostate cancer | Population-based | NA | 132/158 | Age |
| Brankovic et al. 2013 | Serbian | Caucasian | G894T,T-786C | Prostate cancer | Population-based, BPH | PCR-RFLP, CycleSeq Kit | 150/250 | Age |
| Safarinejad et al. 2013 | Iran | Caucasian | G894T,T-786C, 4a/b | Prostate cancer | Population-based | PCR-RFLP | 170/340 | Age |
| Ziaei et al. 2013 | Iran | Caucasian | G894T | Prostate cancer | BPH | Sequencing | 95/111 | Age, BMI |
| Riener et al. 2004 | Austria | Caucasian | G894T,4a/b | Vulvar cancer | Hospital-based | Pyrosequencing, PCR | 68/272 | NA |

Table 2. Stratified Analyses of eNOS Polymorphisms on Cancer Risk

| Variables | N | Allele(T vs G) | | Homozygous(TT vs GG) | | Heterozygous(TG vs GT) | | Dominant(TT+GT vs GG) | | Recessive(TT vs GT+GG) | |
|--------------------|----|--------------------|-------|----------------------|-------|------------------------|-------|-----------------------|-------|------------------------|-------|
| | | OR(95%CI) | Phet | OR(95%CI) | Phet | OR(95%CI) | Phet | OR(95%CI) | Phet | OR(95%CI) | Phet |
| G894T | | | | | | | | | | | |
| Total | 27 | 1.053(0.969-1.143) | 0.002 | 1.178(0.957-1.449) | 0.014 | 1.015(0.946-1.088) | 0.06 | 1.006(0.956-1.060) | 0.977 | 1.105(0.979-1.248) | 0.056 |
| HWE | 23 | 1.081(0.991-1.180) | 0.003 | 1.194(0.974-1.464) | 0.034 | 1.047(0.952-1.152) | 0.119 | 1.017(0.960-1.078) | 0.974 | 1.103(0.974-1.250) | 0.119 |
| Match | 19 | 1.058(0.991-1.128) | 0.296 | 1.219(1.019-1.457) | 0.27 | 1.034(0.940-1.139) | 0.112 | 1.011(0.958-1.068) | 0.949 | 1.128(0.981-1.297) | 0.388 |
| Female | 10 | 1.112(1.000-1.236) | 0.237 | 1.414(1.056-1.892) | 0.2 | 1.017(0.882-1.172) | 0.248 | 1.007(0.925-1.097) | 0.927 | 1.356(1.108-1.661) | 0.14 |
| Ethnicity | | | | | | | | | | | |
| Caucasian | 20 | 1.050(0.956-1.152) | 0.018 | 1.102(0.886-1.370) | 0.025 | 1.045(0.927-1.178) | 0.075 | 1.022(0.950-1.099) | 0.844 | 1.038(0.911-1.182) | 0.12 |
| Asian | 5 | 1.020(0.825-1.262) | 0.055 | 2.074(0.881-4.882) | 0.251 | 1.070(0.684-1.672) | 0.196 | 0.992(0.916-1.075) | 0.937 | 2.103(1.133-3.903) | 0.278 |
| Mixed | 2 | 1.518(0.684-3.369) | 0.011 | 3.691(0.246-55.335) | 0.054 | 0.950(0.769-1.173) | 0.107 | 0.996(0.801-1.238) | 0.537 | 1.648(1.056-2.571) | 0.071 |
| African | 1 | 0.760(0.457-1.265) | 0 | 3.039(0.166-55.566) | 0 | 0.826(0.478-1.428) | 0 | 0.958(0.684-1.343) | 0 | 1.105(0.979-1.248) | 0 |
| Cancer type | | | | | | | | | | | |
| Prostate cancer | 7 | 1.063(0.902-1.253) | 0.118 | 1.061(0.779-1.445) | 0.368 | 1.013(0.890-1.153) | 0.523 | 1.006(0.908-1.116) | 0.988 | 1.043(0.831-1.310) | 0.346 |
| Breast cancer | 7 | 1.097(1.001-1.203) | 0.815 | 1.346(1.012-1.789) | 0.245 | 1.038(0.918-1.174) | 0.575 | 1.015(0.929-1.108) | 0.93 | 1.269(1.028-1.566) | 0.186 |
| Colorectal cancer | 5 | 0.979(0.788-1.216) | 0.003 | 0.916(0.542-1.549) | 0.017 | 1.016(0.889-1.162) | 0.147 | 1.004(0.914-1.103) | 0.858 | 0.944(0.747-1.194) | 0.043 |
| Gastric cancer | 2 | 0.749(0.549-1.021) | 0.308 | 0.390(0.077-1.975) | 0.242 | 0.744(0.519-1.065) | 0.463 | 0.912(0.709-1.173) | 0.446 | 0.424(0.127-1.419) | 0.324 |
| Bladder cancer | 2 | 1.293(0.852-1.961) | 0.126 | 1.858(0.719-4.799) | 0.154 | 1.391(0.959-2.018) | 0.002 | 1.214(0.880-1.674) | 0.014 | 1.171(0.680-2.018) | 0.726 |
| Other cancers | 4 | 1.137(0.663-1.948) | 0.016 | 1.659(0.523-5.256) | 0.082 | 0.835(0.575-1.213) | 0.135 | 0.925(0.689-1.244) | 0.681 | 1.819(0.991-3.341) | 0.081 |
| T-786C | | | | | | | | | | | |
| Total | 11 | 1.135(1.048-1.228) | 0.073 | 1.278(1.045-1.562) | 0.302 | 1.136(1.023-1.261) | 0.111 | 1.159(1.047-1.281) | 0.066 | 1.204(1.003-1.447) | 0.54 |
| HWE | 9 | 1.135(1.046-1.231) | 0.044 | 1.265(1.027-1.558) | 0.173 | 1.146(1.030-1.275) | 0.104 | 1.164(1.050-1.291) | 0.052 | 1.182(0.978-1.429) | 0.401 |
| Match | 8 | 1.183(1.081-1.296) | 0.118 | 1.474(1.146-1.895) | 0.443 | 1.174(1.048-1.316) | 0.108 | 1.200(1.075-1.340) | 0.071 | 1.353(1.068-1.715) | 0.687 |
| Caucasian | 7 | 1.157(1.045-1.280) | 0.073 | 1.286(1.030-1.604) | 0.069 | 1.192(1.025-1.387) | 0.327 | 1.224(1.060-1.413) | 0.158 | 1.188(0.973-1.450) | 0.204 |
| Asian | 3 | 1.112(0.973-1.271) | 0.086 | 1.222(0.715-2.087) | 0.989 | 1.111(0.958-1.290) | 0.045 | 1.118(0.967-1.293) | 0.052 | 1.204(0.706-2.055) | 0.998 |
| Mixed | 1 | 0.988(0.644-1.517) | 0 | 1.329(0.468-3.776) | 0 | 0.712(0.373-1.361) | 0 | 0.788(0.421-1.474) | 0 | 1.642(0.627-4.302) | 0 |
| Cancer type | | | | | | | | | | | |
| Prostate cancer | 3 | 1.508(1.153-1.972) | 0.161 | 1.677(1.127-2.494) | 0.409 | 1.161(0.886-1.522) | 0.04 | 1.258(0.972-1.628) | 0.044 | 1.563(1.088-2.245) | 0.879 |
| Breast cancer | 3 | 1.139(0.998-1.300) | 0.126 | 1.494(1.047-2.130) | 0.336 | 1.091(0.924-1.288) | 0.293 | 1.127(0.960-1.322) | 0.15 | 1.371(0.980-1.918) | 0.479 |
| Colorectal cancer | 3 | 1.073(0.940-1.225) | 0.038 | 0.951(0.674-1.342) | 0.754 | 1.144(0.963-1.359) | 0.052 | 1.133(0.959-1.339) | 0.044 | 0.956(0.708-1.291) | 0.823 |
| Gastric cancer | 1 | 1.476(0.827-2.634) | 0 | 1.631(0.556-4.785) | 0 | 1.480(0.630-3.476) | 0 | 1.532(0.738-3.180) | 0 | 1.483(0.517-4.257) | 0 |
| Bladder cancer | 1 | 1.062(0.777-1.452) | 0 | 0.691(0.263-1.817) | 0 | 1.246(0.836-1.856) | 0 | 1.183(0.803-1.744) | 0 | 0.625(0.242-1.617) | 0 |
| 4a/b | | | | | | | | | | | |
| Total | 12 | 1.061(0.862-1.306) | 0 | 1.607(0.901-2.865) | 0.014 | 1.050(0.857-1.287) | 0.002 | 1.081(0.866-1.349) | 0 | 1.508(0.887-2.567) | 0.01 |
| Match | 9 | 1.212(0.977-1.502) | 0 | 2.302(1.315-4.028) | 0.077 | 1.129(0.905-1.408) | 0.002 | 1.193(0.941-1.511) | 0 | 2.109(1.264-3.518) | 0.147 |
| Female | 4 | 0.864(0.674-1.109) | 0.116 | 0.817(0.343-1.944) | 0.186 | 1.050(0.857-1.287) | 0.417 | 0.917(0.718-1.171) | 0.278 | 0.816(0.456-1.462) | 0.262 |
| Ethnicity | | | | | | | | | | | |
| Caucasian | 9 | 1.087(0.820-1.442) | 0 | 1.489(0.703-3.150) | 0.004 | 1.116(0.848-1.469) | 0.002 | 1.136(0.839-1.537) | 0 | 1.430(0.740-2.767) | 0.004 |
| Asian | 3 | 0.968(0.819-1.144) | 0.592 | 1.702(0.761-3.807) | 0.579 | 0.905(0.753-1.088) | 0.387 | 0.933(0.779-1.117) | 0.465 | 1.728(0.773-3.863) | 0.55 |
| Cancer type | | | | | | | | | | | |
| Prostate cancer | 3 | 1.515(0.975-2.353) | 0.029 | 3.234(0.724-14.443) | 0.018 | 1.338(1.013-1.768) | 0.394 | 1.474(1.002-2.170) | 0.131 | 3.003(0.721-12.50) | 0.025 |
| Breast cancer | 3 | 0.960(0.773-1.194) | 0.291 | 1.181(0.629-2.216) | 0.05 | 0.905(0.700-1.170) | 0.289 | 0.921(0.709-1.197) | 0.259 | 1.206(0.645-2.255) | 0.56 |
| Colorectal cancer | 2 | 1.010(0.833-1.225) | 0.578 | 1.686(0.654-4.343) | 0.298 | 0.949(0.760-1.185) | 0.295 | 0.979(0.795-1.204) | 0.404 | 1.717(0.637-4.631) | 0.279 |
| Other cancers | 4 | 0.934(0.565-1.545) | 0 | 0.876(0.207-3.714) | 0.025 | 1.031(0.575-1.848) | 0.001 | 0.989(0.535-1.829) | 0.404 | 0.970(0.379-2.480) | 0.091 |

*The random-effects model was applied when the P value was more than 0.05. Otherwise, the fixed-effects model was used. N Number of comparisons, OR odds ratio; CI confidence interval, Phet P value of Q-test for the heterogeneity test

The association between T-786C polymorphism and cancer risk

A total of 11 studies involving 4169 cases and 4185 controls examined the association between T-786C polymorphism and cancer risk, with 7 in Caucasians, 3 in Asians, and 1 in mixed population (Brazilians). Unlike G894T, we observed a significant association between T-786C polymorphism and cancer risk (C vs T: OR=1.135, 95%CI=1.048-1.228; CC vs TT: OR=1.278, 95%CI=1.045-1.562; TC vs TT: OR=1.136, 95%CI=1.023-1.261; CC+TC vs TT: OR=1.159, 95%CI=1.047-1.281; CC vs TC+TT: OR=1.204, 95%CI=1.003-1.447). When

stratified by ethnicity, a significant association between T-786C polymorphism and cancer risk was found in Caucasians. When stratified by cancer type, a significantly increased risk was found in prostate cancer (C vs T: OR=1.508, 95%CI=1.153-1.972; CC vs TT: OR=1.677, 95%CI=1.127-2.494; CC vs TC+TT: OR=1.563, 95%CI=1.088-2.245) and breast cancer (CC vs TT: OR=1.494, 95%CI=1.047-2.130) but not in colorectal, gastric, or bladder cancer.

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by sequential omission of each eligible study. The analysis

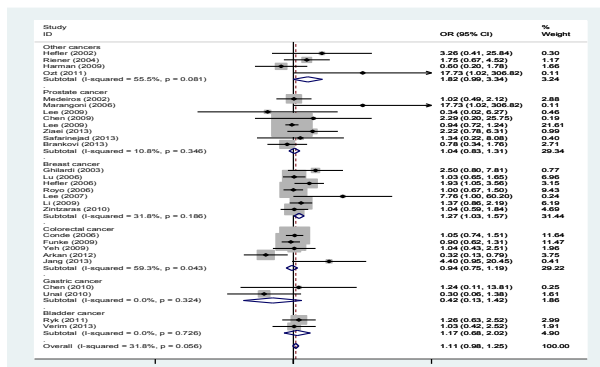


Figure 2. Forest Plot of ORs with a Fixed Effect Model for Association between the eNOS G894T Polymorphism and Overall Cancer Risk under Recessive Model(TT vs GT+GG)

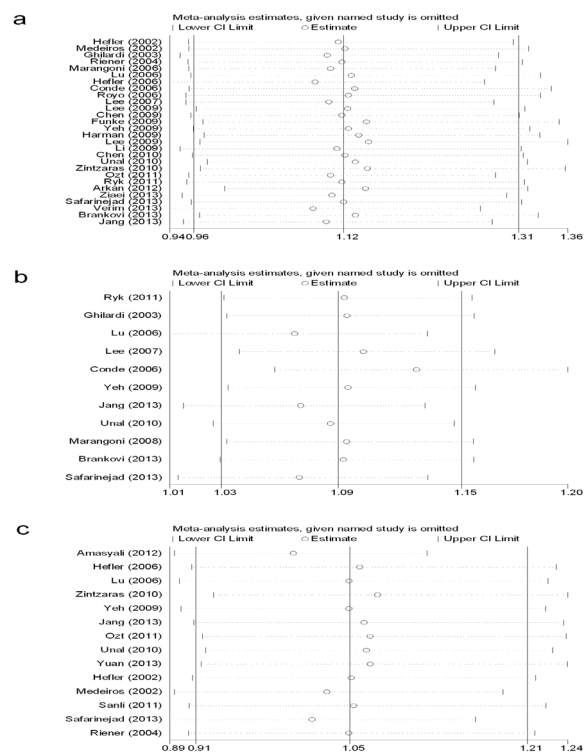


Figure 3. Sensitivity Analysis of Overall OR Coefficients for (a G894T TT vs GG, b T-786C C vs T, c 4a/b aa+ba vs bb). Results were calculated by omitting each study in turn. The two ends of the dotted lines represent the 95%CI

results showed that the pooled ORs were not significantly affected by any individual study (Figure 3b), thus indicating a robust result of the analysis.

Begg's funnel plot was constructed to evaluate the publication bias of literatures on cancer. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Figure 4b). Furthermore, Egger's test provided statistical evidence for the lack of publication bias ($t=0.48, p=0.645$).

The association between 4a/b polymorphism and cancer risk

14 studies with 3430 cases and 3842 controls investigated the association between 4a/b polymorphism and cancer risk. All the studies are in HWE. Increased

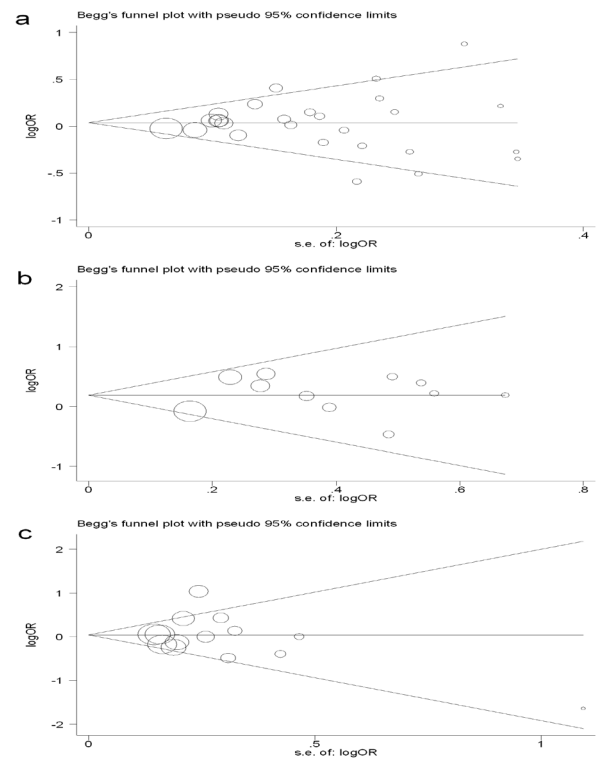


Figure 4. Begg's Funnel Plot for Publication Bias Test. a G894T T vs G, b T-786C CC vs TC+TT, c 4a/b ba vs bb Each point represents a separate study for the indicated association. The circles represent the weight of individual study

cancer risk were detected in studies with matched controls enrolled (aa vs bb: OR=2.302, 95%CI=1.315-4.028; aa vs ba+bb: OR=2.109, 95%CI=1.264-3.518). Subgroup analysis suggests that increased susceptibility was revealed in prostate cancer (ba vs bb: OR=1.338, 95%CI=1.013-1.768; aa+ba vs bb: OR=1.474, 95%CI=1.002-2.170).

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by sequential omission of each eligible study. The analysis results showed that the pooled ORs were not significantly affected by any individual study (Figure 3c), thus indicating a robust result of the analysis.

Begg's funnel plot was constructed to evaluate the publication bias of literatures on cancer. The shape of the funnel plot seemed symmetrical, indicating the absence of publication bias (Figure 4c). Furthermore, Egger's test provided statistical evidence for the lack of publication bias ($t=-0.33, p=0.745$).

Discussion

Recent literature indicates that eNOS can modulate cancer-related events such as angiogenesis, apoptosis, cell cycle, invasion, and metastasis (Ying and Hofseth, 2007). Correlation between eNOS and cancers has been reported (Erdamar et al., 2006; Tu et al., 2006; Yu et al., 2006). Endothelial NOS plays a predominant role in VEGF-induced angiogenesis and vascular permeability (Fukumura et al., 2001). Stress is accepted to constitute a relevant factor in the development of cancer (Reiche et al., 2004). Animal experiments show that eNOS play a pivotal role for eNOS in chronic stress-induced initiation

and promotion of tumour growth (Barbieri et al., 2012). These findings suggest eNOS as a key factor promoting carcinogenesis. Effects of polymorphisms of the eNOS gene on plasma NO concentrations have been reported. The mutant allele of the T-786C and 4a/b polymorphism has been associated with altered eNOS activity and synthesis of NO (Wang et al., 1997; Nakayama et al., 1999). The eNOS polymorphisms might affect the process of carcinogenesis by influencing the expression of eNOS. Till now, many efforts have been made to explore the association between eNOS polymorphisms and cancer risk, whereas the results remain controversial. Here, we conducted a comprehensive meta-analysis to provide a complete picture of the role of eNOS polymorphisms in cancer risk.

By performing meta-analysis with studies involving cases and controls, we didn't find that eNOS G894T polymorphism has an overall association with cancer risk. However, in subgroup analysis, the association was found in females (homozygote comparison and the recessive model) and breast cancer (allele contrast model, homozygote comparison and the recessive model). Three earlier meta-analyses show controversial views for breast cancer (Hao et al., 2010; Yao et al., 2010; Fu et al., 2011). Fu thought Hao's study includes unqualified studies. We agree with Fu's opinion about the overlapping data, but we include Lee's study (Lee et al., 2007) for this meta-analysis. We failed to detect a significant association in other cancer types, which could be partly because the number of included studies for particular cancer type was small. For instance, only two studies discussed the eNOS G894T polymorphism and bladder cancer, and a significant association was found in Verim's research (Verim et al., 2013), but we couldn't find this significance in the pooled OR. We found gender differences in subgroup analysis. Estrogen modulation may explain this phenomenon. It was found that estrogen can activate eNOS via MAP kinase-dependent mechanisms (Chen et al., 1999).

As for eNOS T-786C polymorphism, we observed a significantly increased cancer risk in all genetic models by pooling ORs from 11 studies. In subgroup analysis based on ethnicity, elevated cancer risk was detected in Caucasians in four genetic models, while that was not detected in Asians. The different ethnical background and a small number of studies involving Asians may partially explain this difference. When stratified by cancer type, we found a significant association between T-786C polymorphism and increased risk of prostate cancer in three genetic models. That was detected in only homozygote comparison (TT vs GG) in breast cancer. Besides, no significant association was found in colorectal cancer.

As for the Intron 4 VNTR (4a/b) polymorphism, significant association was only found with cancer risk in studies with matched controls in homozygote comparison (aa vs bb) and recessive comparison (aa+ba vs bb). In subgroup analysis based on cancer type, elevated cancer risk was detected in prostate cancer in two genetic models. Interestingly, the minor allele was a in the most studies, while in the study by Ozt et al. (2011), b was the minor

allele. We think this diversity may result from a selection bias or different ethnicity background.

We do a comprehensive electronic search for all available eligible studies and provided an overview of the association between eNOS polymorphisms and cancer susceptibility. Still, there were some limitations in our meta-analysis. First, sample size in any given cancer was not sufficiently large, resulting in insufficient power to detect a slight effect on a certain type of cancer. Second, most of included studies are of Caucasian, relative small sample size in Asians might cause the inconspicuousness. Third, selection bias might exist given the fact that the genotype distribution deviated from HWE in some studies. Fourth, due to the original data of the eligible studies was unavailable, it was difficult for us to evaluate the roles of some special environmental factors and lifestyles such as diet, alcohol consumption, and smoking status in developing cancer. Fifth, the influence of bias in the present analysis could not be completely excluded because positive results are supposed to be published much more quickly than articles with "negative" results.

In conclusion, our meta-analysis suggested that the eNOS genetic polymorphisms contribute to the susceptibility of cancers. The eNOS T-786C polymorphism is associated with elevated cancer risk. The G894T polymorphism contributes to susceptibility to breast cancer and femals; and the 4a/b polymorphism may be associated with prostate cancer risk. Large well designed epidemiological studies are needed to validate our findings.

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